EXHIBIT 1

Tissue engineering work:

[redacted]

Redacted

Mixes of polymers for testing with chondrocytes

1 Poly(L-Lactic Acid MW 100,000, Polysciences Inc (Cat 18402)=PLA
#2 Poly(L-Lactide Acid-Co-glycolide), Polysciences Inc (Cat16587)=PLGA 70:30
#3 502H (PGA wouldn't go into solution)
#4 Poly gultamate gamma benzene

In triplicate for each mix 32 different mixes max (96well/3) 31 since empty well is one choice.
10:90 , 50:50, 90:10
(need extra wells for no antibody controls, no cell controls, etc)
6 wells w/ nothing
9 of each of the following
1
2
3
4
3 of each of the following (10:90 , 50:50, 90:10 mixes)
1,2
1,3
1,4
2,3
2,4
3,4

make 9 plates: 6 black, 3 white. Each well has a total of 250ug polymer

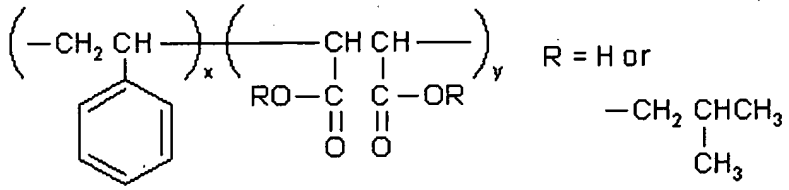
So need starting concentration of 250ug/100ul. 39 wells per plate/ reagent (overestimate).
9 plates so 351 total wells w/ 250ug each sample. Dissolve 90mg of each reagent into 36mls of DMSO

	1	2	3	4	5	6
A	1	1	1	1	1	1
B	2	2	2	2	2	2
C	3	3	3	3	4	4
D	blank	blank	blank	blank	blank	blank
E	1-90%,2-10%	1-90%,2-10%	1-90%,2-10%	1-10%,3-90%	1-10%,3-90%	1-10%,3-90%
F	1-10%,4-90%	1-10%,4-90%	1-10%,4-90%	1-50%,4-50%	1-50%,4-50%	1-50%,4-50%
G	2-50%,3-50%	2-50%,3-50%	2-50%,3-50%	2-90%,3-10%	2-90%,3-10%	2-90%,3-10%
H	2-90%,4-10%	2-90%,4-10%	2-90%,4-10%	3-10%,4-90%	3-10%,4-90%	3-10%,4-90%

06/19/00
Plate experiment 3

Many of the compounds were not soluble in DMSO, but were soluble in chloroform. Also, for each mix, I want 3 data points, control with no primary antibody, no primary or secondary antibody, and no cells. Therefore want 6 wells for each mix. Also, 250ug/well was to thin a film (was web like in microscope). Estimate 2mg/well should be enough.

1 Poly(L-Lactic Acid MW 100,000, Polysciences Inc (Cat 18402)=PLA
#2 502H Poly(L-Lactide Acid-Co-glycolide), MW 20000PLGA 50:50
#3 Poly DL Lactic acid MW 15000-20000 Polysciences Inc
#4 (sigma 435287) soluble in acetone, not chloroform



There are 96 wells, want 6 for each plus 6 wells with no polymer added. 16 available spots
No sample added
1 2 3 4
(10:90 , 50:50, 90:10 mixes)
1,2
1,3
2,3
1,4 (90:10 only)
2,4 (90:10 only)
make 8 black plates and 8 white plates

5x6x2mg/wellx20plates. Dissolve 1200mgs of #1,#2, #3 at a concentration of 2mg/100ul=60ul of chloroform.

12x2mg/wellx20plates (#4). Dissolve 480mg #4 at a concentration of 2mg/100ul into 24ml acetone.

	1	2	3	4	5	6	7	8	9	10	11	12
A	blank	blank	blank	blank	blank	blank	1-10%,3-90%	1-10%,3-90%	1-10%,3-90%	1-10%,3-90%	1-10%,3-90%	1-10%,3-90%
B	1	1	1	1	1	1	1-50%,3-50%	1-50%,3-50%	1-50%,3-50%	1-50%,3-50%	1-50%,3-50%	1-50%,3-50%
C	2	2	2	2	2	2	1-90%,3-10%	1-90%,3-10%	1-90%,3-10%	1-90%,3-10%	1-90%,3-10%	1-90%,3-10%
D	3	3	3	3	3	3	2-10%,3-	2-10%,3-	2-10%,3-	2-10%,3-	2-10%,3-	2-10%,3-

							90% 2-50%,3-50%	90% 2-50%,3-50%	90% 2-50%,3-50%	90% 2-50%,3-50%	90% 2-50%,3-50%	90% 2-50%,3-50%
E	4	4	4	4	4	4	2-90%,3-10%	2-90%,3-10%	2-90%,3-10%	2-90%,3-10%	2-90%,3-10%	2-90%,3-10%
F	1-10%,2-90%	1-10%,2-90%	1-10%,2-90%	1-10%,2-90%	1-10%,2-90%	1-10%,2-90%	1-90%,4-10%	1-90%,4-10%	1-90%,4-10%	1-90%,4-10%	1-90%,4-10%	1-90%,4-10%
G	1-50%,2-50%	1-50%,2-50%	1-50%,2-50%	1-50%,2-50%	1-50%,2-50%	1-50%,2-50%	2-90%,4-10%	2-90%,4-10%	2-90%,4-10%	2-90%,4-10%	2-90%,4-10%	2-90%,4-10%
H	1-90%,2-10%	1-90%,2-10%	1-90%,2-10%	1-90%,2-10%	1-90%,2-10%	1-90%,2-10%	2-90%,4-10%	2-90%,4-10%	2-90%,4-10%	2-90%,4-10%	2-90%,4-10%	2-90%,4-10%

[redacted]

Note: In some plates, wells 6 and 12 were empty, due to lack of material. Also, wells appear to have dried asymmetrically, which may or may not impact reproducibility of some assays.

Plate 20,000 cells/well in 200ul of Tahir's medium. Also, Plate 400,000 cells in .5 ml in 24 well plates for viral transfection experiments. Finally plate 80,000, 40,000, and 20,000 in 96 well clear plates to investigate cell density on tissue-culture polystyrene.

[redacted]

Synthesis and assay of Isothiocyanate conjugation to PLGA polylysine. Using Florescamine assay, determine kinetics of primary amine disappearance.

1) Dissolve 6mg of florescamine into 1950ul DSMO +50ul Triethylamine(3ug/ul or 10.8nmoles/ul)
2) Dissolve 25 mg of polylysine PLGA into 2000ul DMSO (or DMSO+cholorform if necessary). 1.25ug/ul polylysine approximately
2) Dissolve 25mg of PLGA 502H into 2000 ul of DMSO
3) Dissolve 5mg of isothiocyanate sugar into 500ul of DMSO + 30ul Triethylamine
4) Add 50ul of florescamine into 40 wells in a black PP plate (4 deep, 10 across)
5) Add 25ul of each PLGA sample into 2 of the wells
6) Add 250ul of sugar to each PLGA
7) Take 28.1ul time points 1,2,5,10,20,30,1hr,2hr,3hrs (note: take over and read after 30 minutes to get an idea)

[redacted]

Plate nerve cells into microwell plates. 7 plates, ~100 wells, 13,600 cells/ well. Need ~ 20 million cells. Need 100,000 cells/ml ~180 mls. **Lanes 6 and 12 are empty for no-cell controls.** Tommorrow, perform nestin and proliferation assays for both nerve and chondrocytes.

Freeze down 10 vials for each T175cm^2 flask full of cells. Trypsinize w/ 3mls, add 10mls media+10%FBS extra +10% DMSO.

[redacted]

Cell quantitation using CyQUANT Assay (excite 480, read 520).

1) remove media, freeze at –70C, resupsend in the following solution
2) Dilute lysis buffer (component B) 20 fold in DI water. 200ul needed for each so make 41mls. Dilute Component A 400-fold into diluted B (add 100ul GR stock)
3) excite 480, read 520.

Nerve cell results (day after plating cells):

1	2	3	4	5	6	7	8	9	10	
168.89	191.642	177.629	150.382	183.956	5.736	189.14	105.063	99.503	121.381	10(

269.44	125.833	102.964	155.758	161.607	6.601	92.097	124.389	143.168	119.272	91
135.037	117.99	138.724	134.92	137.45	7.217	112.583	111.004	172.838	114.862	84
102.064	88.187	118.264	106.393	103.373	7.228	104.574	94.032	122.455	90.543	76
364.566	166.237	185.949	248.05	215.762	49.948	104.237	105.425	119.15	89.884	85
121.15	127.844	156.24	144.248	173.694	11.695	139.255	114.68	135.921	99.157	135
126.079	159.301	119.844	183.536	165.553	6.482	123.906	103.089	216.139	166.308	369
125.69	189.216	163.92	134.851	205.986	5.398	105.85	120.382	103.139	85.103	82

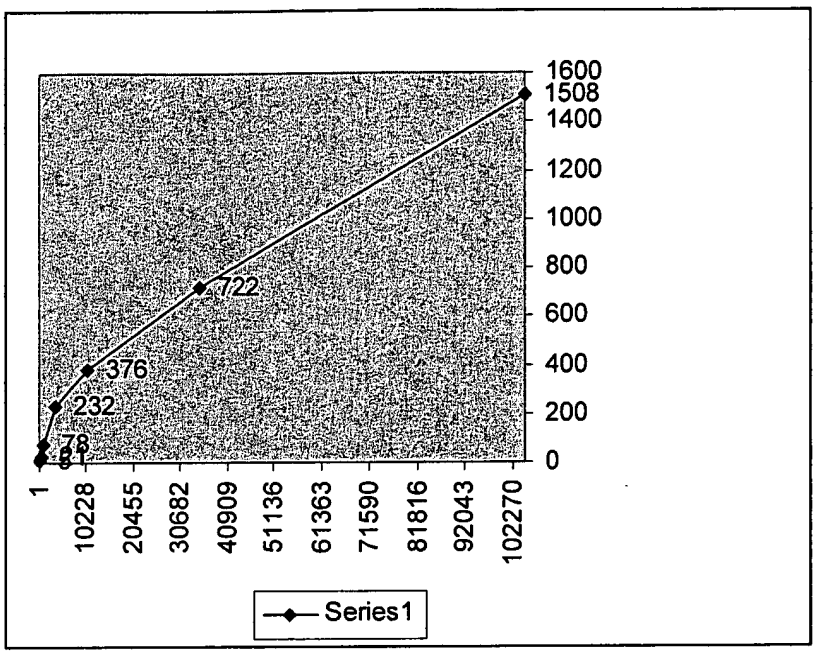
Chondrocytes (grown for 4-5 days).

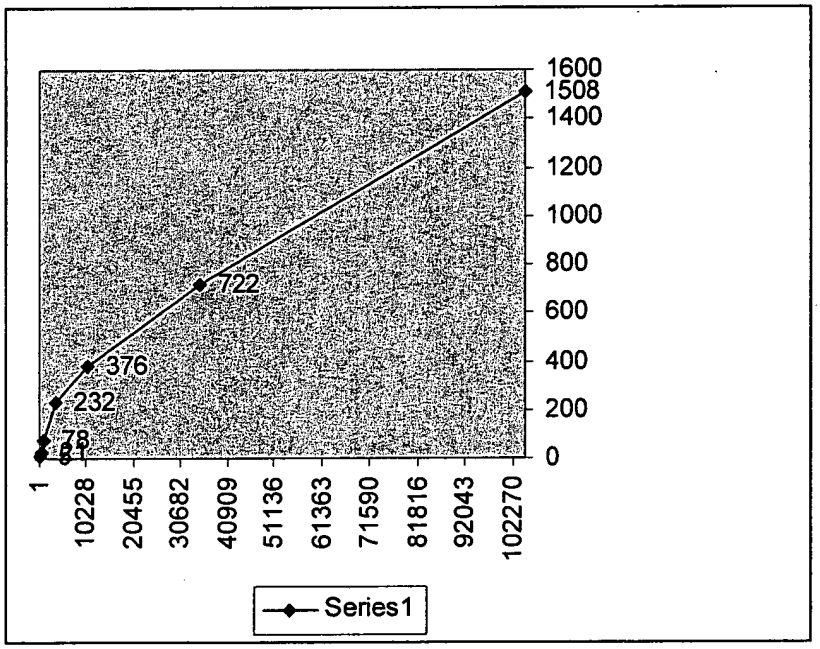
1	2	3	4	5	6	7	8	9	10	
199.598	75.67	74.459	66.238	68.042	73.225	48.484	48.751	47.814	47.353	62
72.356	70.443	58.28	75.431	53.751	50.742	65.822	54.168	49.613	51.77	46
74.323	55.316	92.767	46.538	93.455	94.404	50.126	45.682	39.711	48.394	58
60.363	55.366	44.499	47.915	48.093	39.409	50.505	51.175	37.428	37.299	43
187.204	112.308	127.407	140.677	126.888	97.533	46.949	54.486	33.723	55.036	58
136.908	160.629	145.705	146.052	162.193	110.806	53.871	47.512	64.51	69.201	69
88.964	72.917	72.083	70.996	64.193	59.073	117.376	98.353	100.203	84.239	93
106.161	81.138	71.165	69.34	62.546	61.822	130.643	108.639	112.052	95.879	115

Chondrocytes had white chunks in some of the wells – possibly tissue chunks. Collagen stains should be interesting.

Calibration of cyquant assay:

# of cells									
105000									
35000									
10500									
3500									
1050									
350									
none									
none									
	1452.696	561.196	457.604	210.784	69.398	11.144	3.568	2.998	3.458
	1532.777	844.817	304.607	219.269	66.647	21.947	11.338	7.013	3.35
	1685.34	852.906	624.351	259.54	69.305	16.078	3.606	3.512	3.703
	1291.787	714.517	281.501	262.472	97.025	23.92	3.433	3.958	5.002
	1367.837	645.399	290.702	244.299	83.144	22.314	3.85	3.57	3.867
	1559.616	739.149	234.986	242.69	84.905	22.677	3.754	3.819	3.547
	1762.3	874.04	334.57	199.416	73.63	21.908	3.486	3.593	3.356
	1414.249	540.753	480.581	215.474	77.64	25.502	3.559	3.375	3.741
average	1508.325	721.5971	376.1128	231.743	77.71175	20.68625	4.57425	3.97975	3.753
stdev	149.0121	122.6078	123.5813	22.07666	9.57301	4.410696	2.559619	1.177536	0.50313





[redacted]

Step 1: quantitation of sensitivity for luminescence and flouresence assays:

AP
Using diluted goat anti-mouse antibody, 1:500 in 1.5% goat serum+PBS, add 1,3 10, 30, 100ul into 100ul PBS into microtitre wells. To each add 100, 250, or 500 ul tropix)
Detect luminescence after ~ 10 min and ~20 min.

Flouresence

Using 1:400 diluted antibody in 1.5% goat serum+PBS, add 1,3, 10,30, 100 ul into 100ul PBS into a black plate

Antibody assays for chondrocytes:

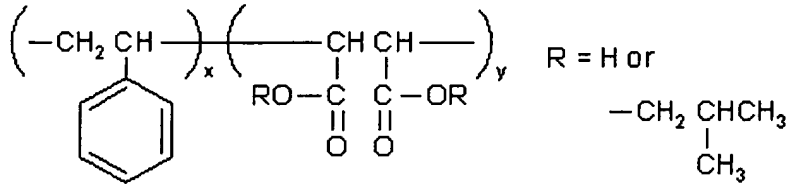
1) Remove media
2) Wash with PBS
3) Add 200ul 10%Formalin for 2-5 minutes
4) Wash with PBS 3 times
5) Add 200ul 0.1% Trition X-100, 10% Goat serum in PBS. Incubate 30'
6) Wash w/ PBS 3 times
7) Add Primary antibody (to collegen 1 – dilute 1:500 or collegen 2 dilute -- 1:5) in 1.5% goat serum+PBS. Add 100ul to each well, incubate for ½ hr
8) Wash w/ PBS 2 times
9) Add 100ul Secondary Anti mouse AP from Zymed (diluted 1:500) or Anti mouse molecular probes in 1.5% goat serum+PBS.
10)

[redacted]

Resuspend NC cells and plate 1) 150,000 per well in 6 well plate containing coverslip 20mls (1,500,000 cells) into a 6cm dish containing a slide w/ polymers 1,2,3,4 (see above). The slide used is "superfrost" cat-ionic slide that appears to retain polymers nicely compared to pure glass.

Note: polypropelene also appeared to retain polymer films nicely in the presence of PBS (note: polymers suspended in chloroform.)

[redacted]**New slide making techniques**
#1 Poly(L-Lactide Acid-Co-glycolide) from Polysciences
#2 502H Poly(L-Lactide Acid-Co-glycolide), MW 20000PLGA 50:50
#3 Poly DL Lactic acid MW 15000-20000 Polysciences Inc
#4 (sigma 435287) soluble in acetone, not chloroform



Dissolved 1gm of 1,2,3 in 3ml chloroform. Dissolved 1gm of #4 in acetone. Add to slides, different spot sizes for each sample. Add drops to: 2, epoxy, 2, epoxy+Teflon tape, 2, superfrost, 2, superfrost+Teflon tape. After drops dry on epoxy 3hr, soak chip in 50% EtOH for ½ hr at room temp..

Then take 1 ml of each and mix w/ 1ml of DMSO and repeat slide procedures w/ DSMO and dry in vac overnight

[redacted] **immunoflouresence of nerve cells on slides:**

Note: perform 2 controls: 1) no primary antibody, 2) no cells (one slide for each). Reconstitute antibody by adding .4ml of DI water, shaking, and let sit for 1-2 hrs at RT. Then add 50% glycerol and aliquot and freeze.

1) Remove media
2) Wash with PBS
3) Soak in 10%Formalin for 2-5 minutes
4) Wash with PBS 3 times
5) Soak in 0.1% Trition X-100, 10% donkey serum in PBS. Incubate 30'
6) Wash w/ PBS 3 times
7) Add Primary antibody to neurofilament – rabbit IgG – dilute 1:200 in 1.5% goat serum+PBS. Make 2ml. Incubate ½ hr
8) Wash w/ PBS 2 times
9) Add Secondary Anti-rabbit Cy3 antibody from Jackson. Dilute 1.5% donkey serum+PBS. Incubate ½ hr. **note: use Erins antibodies this time. (OG anti rabbit)**
10) Wash w/ PBS 3 times

[redacted]

Redacted

[redacted] make media for C17-2 cells

500ml DMEM
50ml iFBS
25ml HS
12.5ml Bicarbonate buffer
5ml pen/strep/fungizone
5ml L-glutamate

[redacted] Couple stuff to plates and test cell growth.

Couple poly lysine, isoleucine, and dextran to xenobind and epoxide slides.

1) dissolve 20mg poly lysine in 5mgs
2) dissolve 400mgs isoleucine in 20mls
3) dissolve 1.1gms dextran in 20mls

pH all to 9-10 by addition of 500mM NaOH (pH determined by pH paper)

Add a large drop of each to slides, and incubate at 37C in a dish w/ water in a tip box (to prevent evaporation), for 2 hr. Remove remaining sample via vaccume, wash with water, then wash in PBS+0.1% Triton X-100. Wash with water, and add to tube with 3% BSA in PBS, or C17 media without serum. Incubate for 2-3 hrs. wash with sterile PBS twice and then plate cells onto slides in 15cm dish.

Results: cells attached to all slides but silanated. Treatment effects were difficult to distinguish. In general, cells looked round and unattached.

[redacted] Polymers on "silanated" amino terminate slides

Slide was washed with chloroform, then DMSO, then chloroform. Spots of PLGA 502H were dropped on the slide (and they seem to stick fairly well -- made by dissolving 250mg of 502H in 1ml of DMF). The slide was washed twice in PBS and then concentrated C17-2 cells were plated. They appear to stick decently to both polymer and slide.

[redacted] attachment of PLGA to slides, then treat w/ modified PEGs

Using previously dissolved 502H AND new polymersciences 350mg in 2ml DMF. Put spots on slide and then dry in house vac. PEGs were pH'd with either HCl or NaOH to ! pH7. these were then incubated with the polymers for ~45'. None of the PEG's affected cell attachment, and in fact it looked like the cells PREFERED the PEG treatment...

[redacted] coating slides w/ polyHEMA and bis amine PEG

Dissolve approximately 1gm of Poly HEMA (p3932) in 100% ethanol. Doesn't dissolve well in either chloroform or DMF. Ethanol solution is slowly dissolving, so rotate overnight. Also, dissolve 100mg of PEG-2 (NH_2) and dissolve in 1ml of DMF.

Add one spot to an epoxy and Xenobind slide and incubate 1hr at room temp. at the other end of the slide, take enough HEMA in EtOH to coat and place on slide. Allow to incubate 1 hr at room temp then 1hr at 37C. Slides were washed with PBS twice, and then cells were added in a 15 cm dish.

PEG didn't keep cells from growing

[redacted] immunofluorescence of chondrocytes cells on arrayed slides:
Bovine Chondrocytes were grown on arrays for 7 days. Cells were seeded into 2 75x25cm dishes, each with 4 slides in each dish. Each dish had one slide with big spots (20nl, 5% solutions) and 3 slides with pin arrayed spots. One observation already – pin arrayed spots seemed to have largely disappeared after 3 days, so next time I will try to layer several spots on top of each other
Note: need to perform 2 controls, but I will only do one : 1) no primary antibody, 2) no cells (one slide for each).

1) Remove media
2) Wash with PBS
3) Soak in 10%Formalin for 2-5 minutes
4) Wash with PBS+1.5% goat serum 1 times
5) Wash with PBS 2 times
6) Soak in 0.1% Trition X-100, 10% goat serum in PBS. Incubate 30'
7) Add Primary antibodies
   Big spots – collegen2 antibody (not diluted)
   Pin array – collegen2 antibody (not diluted)
   -- collegen1 antibody from sigma (1:500)
   dilute in 1.5% goat serum+PBS. Make 1ml. Incubate 2 hrs at room temp
   Also incubate 1 slides with 1.5% goat serum+PBS (for no primary control)

   Note: cells look like they might have partially dried. Bottom of big spots looked ok tho.

8) Wash w/ PBS +1.5% goat serum (10' per washing step) 1 copeland/slide
9) Wash w/ PBS+_1.5% goat serum+.2% Triton X-100
10) Wash w/ PBS +1.5% goat serum
11) Add anti-mouse rhodamine (dilute molecular probes rhodamine 2ndary 1:400). Also add 2ndary to control slides w/ no primary antibody
12) Wash w/ PBS +1.5% goat serum 1 times (10 minutes/ washing step, make sure
13) Wash w/ PBS 3 times (10 minutes/ washing step, make sure
14) Add DAPI fixing solution to slides.

Redacted